

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-5, 7, 13-14, 17-18, 23-27, 29, 31-41, 43-45, 50, 52, 54 and 56 have been amended and claims 8-12, 15-16, 19-22, 30, 46-49, 51, 53 and 55 have been canceled as follows:

Listing of Claims:

Claim 1 (currently amended): A production method of a mammalian artificial chromosome, comprising:
a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising ~~a functional an insertion sequence for specifically inserting a sequence of interest and an insulator sequence~~ into a mammalian host cell;
a second step of selecting transformed cells; and
a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells.

Claim 2 (currently amended): A production method of a mammalian artificial chromosome, comprising:

a first step of introducing a first vector consisting of a yeast artificial chromosome having a mammalian centromere sequence and a mammalian telomere sequence and a second vector

consisting of a yeast artificial chromosome having ~~a functional~~ an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell;

a second step of selecting transformed cells; and

a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells.

Claim 3 (currently amended): The production method according to claim 1 [[or 2]], wherein the first vector has a selection marker gene and the selection of the transformed cells in the second step is carried out by using the selection marker gene.

Claim 4 (currently amended): The production method according to ~~any of claims 1 to 3~~ claim 1, wherein the mammalian centromere sequence comprises a region in which a plurality of the following sequences are arranged at regular intervals:

5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G

Claim 5 (currently amended): The production method according to ~~any of claims 1 to 4~~ claim 1, wherein the mammalian centromere sequence comprises a sequence derived from a human chromosome alpha satellite region.

Claim 6 (original): The production method according to claim 5, wherein the mammalian centromere sequence comprises a 11mer repeat unit derived from a human chromosome 21.

Claim 7 (currently amended): The production method according to ~~any of claims 1 to 6~~
claim 1, wherein the size of the mammalian centromere sequence is about 50 kb or less.

Claim 8 (cancelled)

Claim 9 (cancelled)

Claim 10 (cancelled)

Claim 11 (cancelled)

Claim 12 (cancelled)

Claim 13 (currently amended): The production method according to ~~claim 12~~ claim 1, wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained by partial modification of a loxP site or a FRT site and has a function for inserting the sequence of interest.

Claim 14 (currently amended): The production method according to ~~any of claims 1 to 13~~ claim 1, wherein the quantity ratio of the first vector to the second vector, which are inserted in the first step, is in the range from about 10 : 1 molecular ratio to about 1 : 10 molecular ratio.

Claim 15 (cancelled)

Claim 16 (cancelled)

Claim 17 (currently amended): A mammalian artificial chromosome obtainable by the production method described in ~~any of claims 1 to 16~~ claim 1, which comprises a mammalian replication origin, a mammalian centromere sequence and a functional ~~an insertion sequence for specifically inserting a sequence of interest and an insulator~~ sequence; and

which is circular in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

Claim 18 (currently amended): A mammalian artificial chromosome obtainable by the production method described in ~~any of claims 1 to 16~~ claim 1, which comprises a mammalian replication origin, a mammalian centromere sequence, a mammalian telomere sequence, and a functional ~~an insertion sequence for specifically inserting a sequence of interest and an insulator sequence; encoding a target gene and a regulatory region~~

thereof; and

which is linear in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

Claim 19 (cancelled)

Claim 20 (canceled)

Claim 21 (canceled)

Claim 22 (canceled)

Claim 23 (currently amended): A mammalian artificial chromosome, which comprises a mammalian replication origin, a mammalian centromere sequence, [[and]] an insertion sequence for specifically inserting a sequence of interest[,]] and an insulator sequence, and which is circular in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

Claim 24 (currently amended): A mammalian artificial chromosome,

mammalian telomere sequence, [[and]] an insertion sequence for specifically inserting a sequence of interest and an insulator sequence,

which is linear in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

Claim 25 (currently amended): The mammalian artificial chromosome according to claim 23 [[or 24]], wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained by partial modification of a loxP site or a FRT site and has a function for inserting the sequence of interest.

Claim 26 (currently amended): The mammalian artificial chromosome according to ~~any of claims 17 to 25~~ claim 17, wherein the mammalian centromere sequence comprises a region in which a plurality of the following sequences are arranged at regular intervals:

5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G.

Claim 27 (currently amended): The mammalian artificial chromosome according to ~~any of claims 17 to 25~~ claim 17, wherein the mammalian centromere sequence comprises a sequence derived from a human chromosome alpha satellite region.

Claim 28 (original): The mammalian artificial chromosome according to claim 27, wherein the mammalian centromere sequence comprises an 11mer repeat unit derived from a human chromosome 21.

Claim 29 (currently amended): The mammalian artificial chromosome according to ~~any of claims 17 to 28~~ claim 17, comprising a plurality of the functional sequences or the insertion sequences.

Claim 30 (cancelled)

Claim 31 (currently amended): A mammalian cell containing the mammalian artificial chromosome described in ~~any of claims 17 to 30~~ claim 17 outside the autonomous chromosome.

Claim 32 (currently amended): A human cell containing the mammalian artificial chromosome described in ~~any of claims 17 to 30~~ claim 17 outside the autonomous chromosome.

Claim 33 (currently amended): An embryonic stem cell containing the mammalian artificial chromosome described in ~~any of claims 17 to 30~~ claim 17 outside the autonomous chromosome.

Claim 34 (currently amended): A production method of a mammalian cell in which the functional sequence or the insertion sequence is introduced in a state in which they can be maintained stably for a long term, the method comprising:

introducing the mammalian artificial chromosome obtained by the production method described in ~~any of claims 1 to 16~~ claim 1 or the mammalian artificial chromosome described in ~~any of claims 17 to 30~~ claim 17 into mammalian cells as target cells.

Claim 35 (currently amended): A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising ~~a functional an insertion sequence for specifically inserting a sequence of interest and an insulator~~ sequence into mammalian host cells;

a second step of selecting transformed cells;

a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;

a fourth step of isolating the mammalian artificial chromosome from the selected cells; and

a fifth step of introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

Claim 36 (currently amended): A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

- a first step of introducing a first vector consisting of a yeast artificial chromosome having a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome having ~~a functional~~ an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- a second step of selecting transformed cells;
- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- a fourth step of isolating the mammalian artificial chromosome from the selected cell; and
- a fifth step of introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

Claim 37 (currently amended): A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

- a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising ~~a functional~~ an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- a second step of selecting transformed cells;

- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- a fourth step of fusing the selected cell with a mammalian cell having an ability of forming micro-cells;
- a fifth step of selecting a hybrid cell capable of forming micro-cells and containing the mammalian artificial chromosome; and
- a sixth step of forming micro-cells from the selected hybrid cell.

Claim 38 (currently amended): A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

- a first step of introducing a first vector consisting of a yeast artificial chromosome including a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome including ~~a functional~~ an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- a second step of selecting transformed cells;
- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- a fourth step of fusing the selected cell with a mammalian cell having an ability of forming micro-cells;
- a fifth step of selecting a hybrid cell having an ability of forming micro-cells and containing a mammalian artificial chromosome; and

a sixth step of forming micro-cells from the selected hybrid cell.

Claim 39 (currently amended): A production method of mammalian cells containing a mammalian artificial chromosome, comprising:

fusing the micro-cell obtainable by the production method described in claim 37 [[or 38]] with a mammalian cell as a target cell.

Claim 40 (currently amended): A production method of a mammalian cell containing a mammalian artificial chromosome, comprising:

isolating the mammalian artificial chromosome from the host cell containing the mammalian artificial chromosome described in ~~any of claims 17 to 30~~ claim 17; and

introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

Claim 41 (currently amended): A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

fusing a host cell containing the mammalian artificial chromosome described in ~~any of claims 17 to 30~~ claim 17 and a mammalian cell having an ability of forming micro-cells;

selecting a hybrid cell having an ability of forming micro-cells and containing the mammalian artificial chromosome; and

forming micro-cells from the selected hybrid cells.

Claim 42 (original): A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

fusing the micro-cell obtainable by the production method described in claim 41 with a mammalian cell as a target.

Claim 43 (currently amended): The production method of a mammalian cell according to ~~any of claims 34, 35, 36, 39, 40 and 42~~ claim 34, wherein the mammalian cell as a target cell is an embryonic stem cell, embryonic germ cell, or tissue stem cell.

Claim 44 (currently amended): The production method of a mammalian cell according to ~~any of claims 34, 35, 36, 39, 40 and 42~~ claim 34, wherein the mammalian cell as a target cell is formed by inducing an embryonic stem cell, embryonic germ cell, or tissue stem cell so as to be differentiated to a cell of specific tissue.

Claim 45 (currently amended): The production method of a mammalian cell according to ~~any of claims 34, 35, 36, 39, 40 and 42~~ claim 34, wherein the mammalian cell as a target cell is a fertilized egg.

Claim 46 (cancelled)

Claim 47 (cancelled)

Claim 48 (cancelled)

Claim 49 (cancelled)

Claim 50 (original): A vector used for producing a mammalian artificial chromosome, comprising: a sequence of a loxP site or FRT site, or a sequence obtainable by partial modification of a loxP site or FRT site, the sequence having a function for inserting the sequence of interest, and an insulator sequence.

Claim 51 (cancelled)

Claim 52 (currently amended): [[The]] A non-human transformed animal according to claim 51, wherein into which the mammalian artificial chromosome is a mammalian artificial chromosome described in any of claims 17 to 19 claim 17 is introduced.

Claim 53 (cancelled)

Claim 54 (currently amended): [[The]] An XO type mouse embryonic stem cell according to claim 53, wherein the into which a mammalian artificial chromosome is a mammalian artificial chromosome described in any of claims 17 to 19 claim 17 is introduced.

Claim 55 (cancelled)

Claim 56 (currently amended): [[The]] A female chimeric mouse according to claim 55,
wherein the into which a mammalian artificial chromosome is a mammalian artificial chromosome
described in any of claims 17 to 19 claim 17 is introduced.